

Herbivore specific induction of silica-based plant defences

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1 **Herbivore specific induction of silica-based plant defences**

2

3 Running title: Herbivore induced silica defences

4

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15

1 **Abstract**

2 Induced plant responses to herbivory have major impacts on herbivore feeding behaviour,
3 performance and population dynamics. These effects are well established for chemical defences,
4 but induction of physical defences remains far less studied. However, for many plants it is
5 physical defences that play the major role in regulating the levels of herbivore damage sustained.
6 We provide evidence that in grasses induction of physical defences is both specific to herbivore
7 feeding, as opposed to mechanical damage, and is may be dependant on the amount of damage
8 imposed. Furthermore, we show that the magnitude of the induction response is sufficient to deter
9 further damage and affect herbivore performance. We compared silica induction in two grass
10 species in response to vertebrate and invertebrate damage, and to mechanical defoliation.
11 Induction was assessed at two levels of damage over 16 months. Foliar silica content did not
12 increase in response to mechanical defoliation, but damage by either voles or locusts resulted
13 increases in silica content of over 400%. This increase deterred feeding by both voles and locusts.
14 Silica induction in grasses due to repeated damage events over a prolonged period suggests a
15 possible role for silica defence in the cyclical population fluctuations observed in many grass-
16 feeding herbivores.

17

18 **Key words:** antiherbivore defences, feeding preference, induction, *Microtus*, physical defences,
19 phytoliths, *Schistocerca*.

1 **Introduction**

2 Induction of defences in response to herbivore damage is widely recognized as an effective plant
3 defence strategy; particularly in cases where defences are costly or the threat of herbivore attack
4 is intermittent and predictable from previous damage (Karban and Baldwin 1997; Karban et al.
5 1999). Such defences can have profound impacts on the feeding behaviour, growth and
6 population dynamics of herbivores (Haukioja 1991; Underwood 1999). Much previous work has
7 focused on the induction of chemical defences, and on the induction strategies of woody species,
8 with induction thought to be of less significance for grasses, where levels of chemical defences
9 are often low. Physical defences such as silica are considered more important than chemical
10 defences in deterring herbivory in grasses (McNaughton and Tarrant 1983, Vicari and Bazely
11 1993), but little is known about the induction of these physical defences or the effectiveness of
12 such induction against herbivores. Silica has been shown to deter feeding by both vertebrate and
13 invertebrate herbivores (Gali-Muhtasib et al. 1992; Massey et al. 2006; Massey and Hartley
14 2006). Further, it is known to reduce foliage digestibility and hence, to reduce the growth rates of
15 both insect and mammalian herbivores (Massey et al. 2006; Massey and Hartley 2006). Silica is
16 central to the interactions between grasses and grazers (McNaughton 1984; Jernvall and Fortelius
17 2002; Prasad et al. 2005), but almost nothing is known about the magnitude of silica induction in
18 response to herbivory, its effects on subsequent feeding, and hence its effectiveness as an induced
19 defence strategy in grasses.

20
21 To date, only two studies have found evidence for induction of silica (McNaughton and Tarrant
22 1983; McNaughton et al. 1985). This may be due to the fact that most studies examining potential
23 damage-induced silica deposition in grass leaves have tested induction in response to mechanical
24 damage (McNaughton and Tarrant 1983; McNaughton et al. 1985; Brizuela et al. 1986; Cid et
25 al. 1989; Cid et al. 1990; Banuelos and Obeso 2000). It has long been known that artificial
26 damage can elicit different induction responses to those of herbivory (Hartley and Lawton 1987;

1 Hartley and Lawton 1991). Where feeding by herbivores has been considered, studies have relied
2 on correlative evidence, such as comparing elevated silica levels in grasses from areas with high
3 and low grazing pressure (McNaughton et al. 1985; Brizuela et al. 1986). In addition, for silica
4 induction to be an effective resistance mechanism it must deter further feeding by herbivores, but
5 no previous studies have tested the effects of induced silica levels on subsequent herbivore
6 feeding.

7
8 In this study, we compared the effects of herbivory, both insect and mammalian, and mechanical
9 defoliation, on the concentration of silica in the leaves of two perennial grass species. As well as
10 the type of damage, the intensity and timing of damage is also known to influence the magnitude
11 of induction (Underwood 2000; Högestedt et al. 2005). Hence, we measured silica induction in
12 response to a single damage event and to repeated damage events. In addition, we investigated
13 the significance of silica induction for defence through a series of feeding preference trials using
14 both invertebrate and vertebrate herbivores. The study addressed three key questions:

- 15 i. Do different types of damage (mechanical, vertebrate and invertebrate herbivory)
16 induce silica to the same extent? We predicted, as with chemical defences, that silica
17 induction response would be greater following damage by herbivores than after
18 mechanical damage.
- 19 ii. Is induction dependent on the degree of damage sustained? We predicted that
20 continuous damage would elicit a greater induction response than a single damage
21 event.
- 22 iii. Does silica induction deter further herbivore feeding? For silica induction to be an
23 effective defence, the increased levels of silica should deter herbivory.

24

25 **Materials and methods**

26 Study organisms

1 We used two grass species with differing palatabilities and growth rates to test our hypotheses.
2 *Lolium perenne* L. (perennial rye grass) is a relatively fast growing species (77.6 mg DM d⁻¹)
3 with relatively high levels of foliar nitrogen (2.59% DM) and high palatability to grazing
4 mammals. *Festuca ovina* L. (sheep's fescue) is a slower growing species (10.5 mg DM d⁻¹) with
5 lower levels of foliar nitrogen (1.65% DM) and is less palatable than *L. perenne* (Massey et al.
6 2006). Our chosen mammalian herbivore, field vole (*Microtus agrestis* L.), is abundant in
7 grasslands across Northern Europe (Corbet and Harris 1991). While considered a generalist
8 herbivore, feeding primarily on leaves and stems of grasses, voles display highly selective
9 feeding both between and within host plant species, dependent upon levels of nutrients and
10 defences (Marquis and Batzli 1989; Hartley et al. 1995; Hjalten et al. 1996). The voles used in
11 this study came from a captive bred colony at the University of Sussex. We used locusts
12 (*Schistocerca gregaria* Forskal) as our generalist insect herbivore (Raubenheimer and Simpson
13 2003) and nymphs were supplied by a local pet shop. Individual herbivores were used in a single
14 feeding trial and different individuals were used in the feeding trials and to impose damage
15 treatments.

16

17 Experimental design

18 Seeds of *F. ovina* and *L. perenne* were sown onto the surface of compost (John Innes No. 2) in
19 15-cm pots and grown under greenhouse conditions (16/8 h l/d photoperiods, 15-25°C) and
20 watered *ad libitum* for 3 months before the experiments. Plants of each species were randomly
21 allocated into one of two experiments (see below), each of which had four damage treatments (n
22 = 10): **1.** mechanical defoliation (leaves were cut to 5 cm above ground level using scissors); **2.**
23 locust grazing (plants were placed in a locust cage with ten 3rd and 4th instar locust nymphs until
24 they were eaten to approximately 5 cm above ground level. This took approximately 24 hours); **3.**
25 vole grazing (plants were placed in a cage with a single vole until they were eaten to

1 approximately 5 cm above ground level. This took approximately 5 hours); 4. no damage (control
2 plants).

3
4 We simultaneously conducted two experiments to assess the plant's response at different levels of
5 herbivore damage. In experiment 1, plants were subjected to repeated damage treatments, as
6 described above, every 3-4 weeks from 3 months to 15 months old (16 damage events in total). In
7 experiment 2, plants were grown undamaged for 15 months, after which point they sustained a
8 single damage event, as described above. In both experiments, plants were left undamaged for 6
9 weeks following the final damage event to allow regrowth, before removing samples for silica
10 analysis and to conduct feeding preference tests. Plants were treated with a liquid fertilizer after 9
11 months (100 ml 12:1:1 N:P:K, Evergreen lawn food) to maintain nutrient levels in the compost.

12
13 Foliar silica content ($n = 10$) was determined by fusing oven-dried leaf samples (approximately
14 0.2 g) in sodium hydroxide followed by analysis using the colorimetric silicomolybdate technique
15 (Allen 1989).

16
17 Intraspecific feeding preference trials were conducted with plants from all damage treatments
18 using both voles and locusts ($n = 10$). Individual tillers were removed at the base with scissors,
19 matched for size across damage treatments (three tillers per damage treatment per trial) and the
20 leaf area of each tiller scanned (AM-200 leaf area meter, ADC). The base of each tiller was
21 wrapped in moist cotton wool, placed in an Eppendorf tube and arranged randomly in a grid
22 design (3×4 tillers). The grid of tillers was then placed in an insect-tight enclosure with sawdust
23 added level to the base of tillers, so that herbivores could move freely between grasses. Two 4th
24 instar locust nymphs or a single vole were then placed in the cage and left for 6-12 hours at 25°C
25 for locusts or 1-2 hours at 19°C for voles, until approximately 50% of total leaf area was eaten,
26 after which the remaining leaf area of each tiller was measured.

1 Statistical analysis

2 Foliar silica content between species, damage frequencies and damage treatments were compared
3 using a 3-way ANOVA with Tukey's *post-hoc* analysis. In the feeding trials, the leaf area
4 removed from all three tillers per damage treatment was pooled to calculate the total leaf area
5 eaten per damage treatment per trial. Feeding preferences were then compared using mixed-
6 model ANOVA's, in which damage treatment was treated as a fixed factor and trial as a random
7 factor. In this analysis, the only source of variation which can be used as an error term for the
8 calculation of *F*-ratios is the interaction term between the fixed factor and the random factor. This
9 term has 27 degrees of freedom for the treatment factor. There was no error term for the trial
10 factor.

11

12 **Results**

13 Response to damage: foliar silica concentration

14 Single damage events, by either clipping or herbivore grazing, did not alter the silica
15 concentration in the re-growth tissue of either species (Table 1, Fig. 1). Repeated mechanical
16 damage events induced a 129% increase in silica concentrations of *F. ovina* leaves, but there was
17 no significant change in foliar silica concentrations in *L. perenne*. Repeated damage events by
18 both locusts and voles resulted in large increases in silica levels for both grass species (Table 1,
19 Fig. 1). Feeding by locusts resulted in 534% and 399% increases in silica concentrations of *F.*
20 *ovina* and *L. perenne* leaves respectively, while vole feeding resulted in 399% and 414%
21 increases in silica concentrations.

22

23 Feeding preference trials

24 The feeding preferences of both voles and locusts reflected the large increases in silica levels
25 induced by previous herbivore damage. Feeding trials with locusts revealed higher feeding on
26 undamaged *F. ovina* plants compared to all damage treatments (damage treatment, $F_{3,27} = 13.10$,

1 $P < 0.001$; Fig. 2a), and higher feeding on both undamaged and mechanically damaged plants
2 compared with herbivore damaged plants of *L. perenne* (damage treatment, $F_{3,27} = 9.56$, $P <$
3 0.001 ; Fig. 2b). Voles also displayed feeding preference for undamaged or mechanically
4 damaged plants over herbivore damaged plants for both species (*F. ovina*: damage treatment,
5 $F_{3,27} = 5.95$, $P = 0.003$; *L. perenne*: damage treatment, $F_{3,27} = 8.28$, $P < 0.001$; Fig. 2).
6
7 Single damage events did not result in significant changes in the feeding preferences of either
8 locusts (*F. ovina*: damage treatment, $F_{3,27} = 2.77$, $P = 0.061$; *L. perenne*: damage treatment, $F_{3,27}$
9 $= 1.68$, $P = 0.195$; Fig. 2) or voles on plants of either species (*F. ovina*: damage treatment, $F_{3,27} =$
10 1.13 , $P = 0.356$; *L. perenne*: damage treatment, $F_{3,27} = 0.35$, $P = 0.786$; Fig. 2).
11

12 **Discussion**

13 Silica induction in grasses was dependent on both the degree and type of damage sustained. Both
14 grass species responded to locust and vole grazing with induced silica levels, but mechanical
15 damage did not elicit an induction response. Repeated damage events by either insect or
16 mammalian herbivores resulted in large increases in the foliar silica levels of both species
17 (~400% greater than undamaged plants), whereas single damage events were not sufficient to
18 result in induction responses in either grass species. This is the first study to impose actual
19 herbivore damage on grasses and measure the subsequent induction of silica in a manipulative
20 experiment. All previous studies on this subject either correlated silica content with natural
21 damage levels or assessed induction after mechanical damage (McNaughton and Tarrant 1983;
22 McNaughton et al. 1985; Brizuela et al. 1986; Cid et al. 1989; Cid et al. 1990; Banuelos and
23 Obeso 2000) and the degree of induction in our experiment using repeated herbivore damage was
24 much higher than found these previous studies.
25

1 The patterns of silica induction in response to different damage types are consistent with those of
2 many previous studies assessing chemical induction responses. Natural herbivory has been
3 demonstrated to elicit greater induction of chemical defences than mechanical wounding in many
4 systems (Hartley and Firn 1989; Hartley and Lawton 1991; Hamhimaki and Senn 1992, Korth
5 and Dixon 1997; Lawrence and Novak 2004; Pontoppidan et al. 2005). Herbivores activate
6 specific responses at the biochemical and molecular level (Korth and Dixon 1997; Felton and
7 Eichenseer 1999; Reymond et al. 2000), and in insects, oral secretions have been identified as the
8 sources of the cues leading to the unique responses to herbivory (Alborn et al. 1997). Our results
9 suggest that similar response pathways, specific to damage by herbivores, may exist for silica
10 defence induction.

11

12 As our study assessed the impact of silica induction on subsequent herbivore feeding, we provide
13 strong evidence that silica induction is a defence response following damage. The levels of
14 feeding by both voles and locusts were reduced on plants previously damaged by herbivores
15 compared with undamaged and mechanically damaged plants. Previously, the growth and
16 digestion efficiency of locusts and voles have been found to be substantially reduced by high
17 silica levels in *F. ovina* and *L. perenne*, similar to those induced by the herbivore damage
18 treatments (Massey et al. 2006; Massey and Hartley 2006). Therefore, our results suggest a
19 positive feedback mechanism for grass defence, whereby herbivory elicits silica induction in
20 grass leaves, deterring further feeding and potentially reducing growth rates of herbivores.

21

22 The induction response is much greater following repeated damage events than a single damage
23 event, which may be due to a requirement either for several damage events and/or a significant
24 time period to elapse before silica concentration in new leaves increases. These aspects of the
25 feedback between grazers and grasses could be of particular significance to herbivore population
26 dynamics (Lindroth and Batzli 1986; Haukioja 1991; Högestedt et al. 2005). Many herbivore

1 populations undergo cyclical fluctuations, but the delayed density dependent factors underpinning
2 these cycles remain a topic of intense debate (Agrell et al. 1995; Krebs et al. 1995; Klemola et al.
3 2000; Turchin and Hanski 2001). Although predation (Krebs et al. 1995) and parasitism (Hudson
4 et al. 1998) appear to have strong influences in some of these cycles, changes to plant food
5 quality with increased grazing pressure may also play a role (Agrell et al. 1995, Högstedt et al.
6 2005). A recent study compared population cycles of three mammalian (*Microtus agrestis*: field
7 voles, *Lemmus lemmus*: lemmings and *Ovis aries*: Soay sheep) and two invertebrate (*Melolontha*
8 *hippocastani* and *Cerapteryx graminis*) grass feeding herbivores and concluded that changes in
9 food plant quality could dictate the 3-5 year population cycles by determining the duration of the
10 induction response (Högstedt et al. 2005). Previous studies on cyclic dynamics of grass feeding
11 herbivores have failed to identify any factors in grasses which are both affected by herbivore
12 feeding and could influence food quality (Lindroth and Batzli 1986; Bergeron and Jodoin 1993;
13 Klemola et al. 2000). However, none of these studies measured changes in silica concentrations
14 and our study suggests that silica induction could be this key factor. The grazing pressure
15 associated with high population densities could lead to the delayed induction of silica defences.
16 This would deter herbivore feeding and as we have previously shown, lead to reductions in
17 herbivore growth rates and digestion efficiency (Massey and Hartley 2006). This may have direct
18 or indirect effects on herbivore mortality and so could contribute to population change at local
19 scales.

20
21 The variation in silica concentrations between damage treatments and degrees of damage suggest
22 a cost associated with silica defence preventing the provisioning of leaves under all
23 circumstances. Unlike many carbon or nitrogen based antiherbivore defences, where resources
24 involved could be allocated either to growth or defence (Bryant et al. 1983), silica is not directly
25 associated with plant growth (Raven 2003); therefore, the cost of defence is unclear. In addition,
26 more research is required to determine whether induction is a result of increased silica uptake

1 from the soil, increased deposition of phytoliths within leaves, or both of these factors. There is
2 evidence to suggest that both of these explanations are possible: McNaughton et al. (1985) found
3 the highest levels of silica in the roots of grasses; damage by herbivores may result in the
4 reallocation of this silica. Also, many grass species, including *L. perenne*, are known to actively
5 take up silica from the soil (Jarvis 1987; Raven 2003) and herbivore damage could elicit an
6 increase in this active transport.

7
8 We have demonstrated that silica concentrations in grass leaves are induced in response to
9 herbivore damage and that the elevated silica concentrations have deterrent effects on subsequent
10 feeding by both vertebrate and invertebrate herbivores. This evidence for silica induction in
11 grasses is consistent with an evolved defence mechanism and the large increases in induced foliar
12 silica by herbivores have significant implications for the understanding of grass-grazer
13 interactions. Further study is required to determine the extent to which silica induction in grasses
14 affects plant and herbivore performance under field conditions, as well as any role silica may play
15 in the population dynamics of grass feeding herbivores.

16

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22

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1 Table 1: ANOVA results for the effects of damage treatment (undamaged control, mechanical
 2 clipping, locust and vole feeding) and the degree of damage sustained (long-term [experiment 1],
 3 and short-term [experiment 2]) on the silica content of the leaves of two grass species (*F. ovina*
 4 and *L. perenne*).

5

Factor	<i>df</i>	MS	<i>F</i> ratio	<i>P</i> value
Species (S)	1	5.51	10.62	0.001
Degree of damage (D)	1	92.45	178.25	<0.001
Damage treatment (T)	3	22.69	43.74	<0.001
S × D	1	0.30	0.58	0.446
S × T	3	2.27	4.38	0.006
D × T	3	17.37	33.49	<0.001
S × D × T	3	1.10	2.13	0.099
Error	144	0.52		

1 **Figure legends**

2 **Fig. 1** Foliar silica concentration of re-growth tissue of *F. ovina* and *L. perenne* plants following
3 repeated and single damage treatments by mechanical defoliation, locust grazing and vole
4 grazing, with undamaged controls. Values are means (\pm SE). Different letters indicate significant
5 differences between damage treatments (Tukey's test $P < 0.05$).

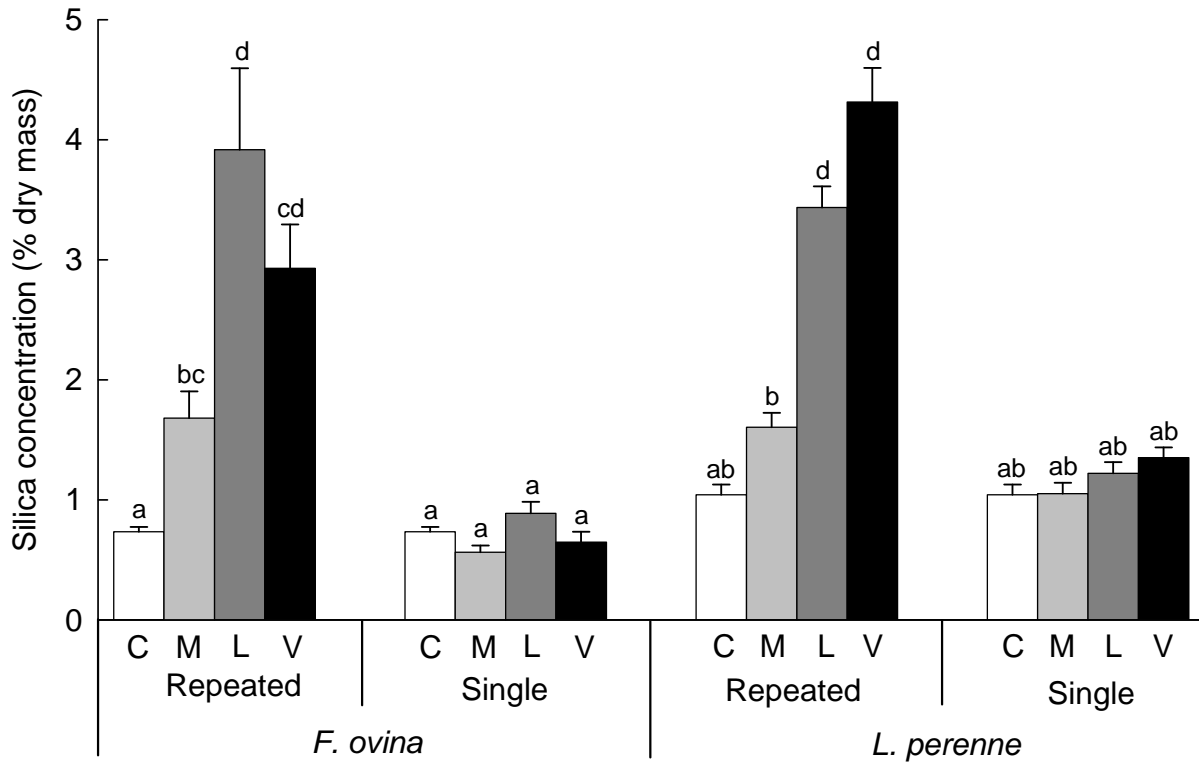
6

7 **Fig. 2** Locust and vole feeding preferences for **a** *F. ovina* and **b** *L. perenne* plants after repeated
8 and single damage events by mechanical defoliation, locust grazing and vole grazing, with
9 undamaged controls. Values are means (\pm SE). Different letters indicate significant differences
10 between damage treatments within each feeding trial (Tukey's test $P < 0.05$), ns = not significant.

1 **Fig. 1**

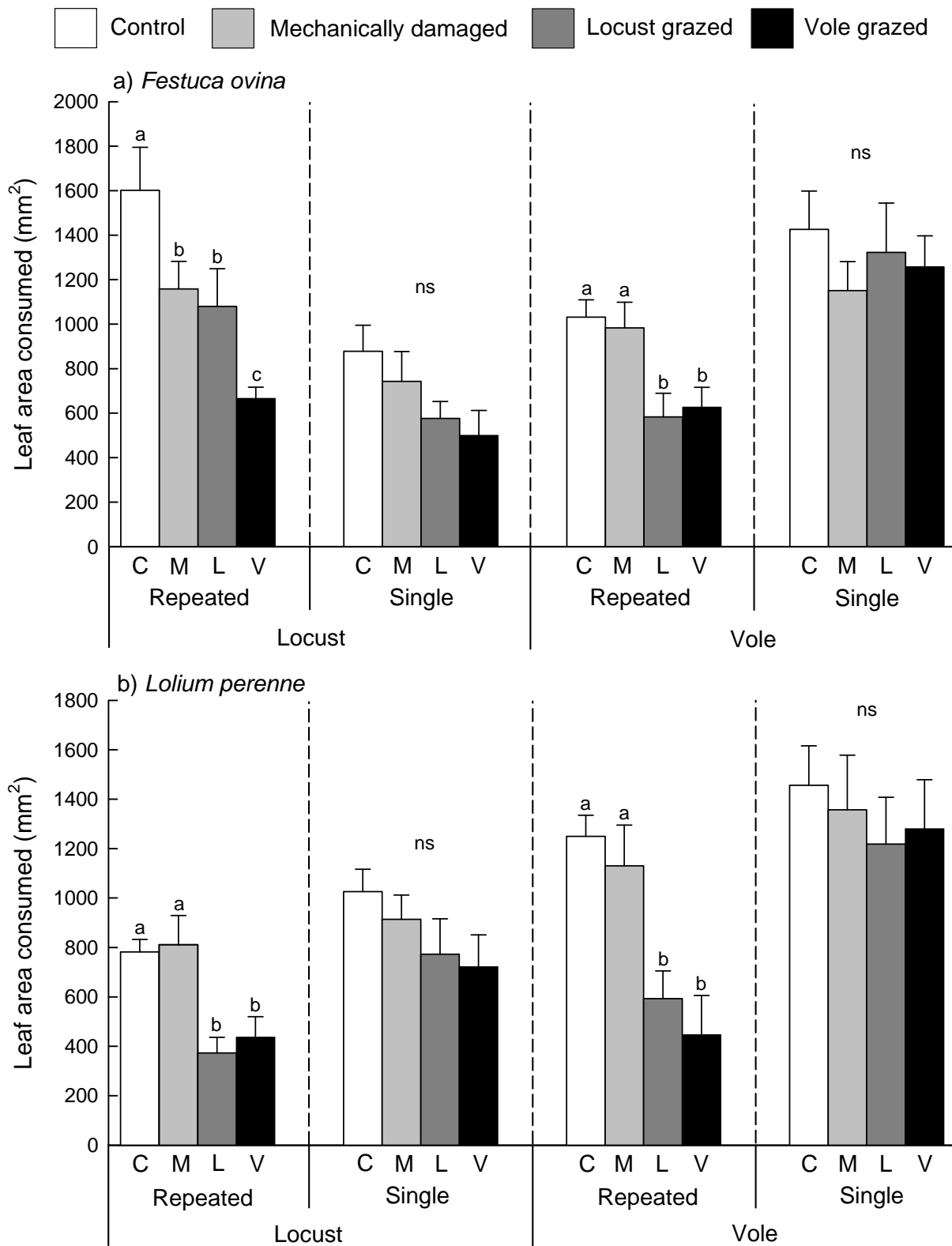
2

Control Mechanically damaged Locust grazed Vole grazed



3

1 **Fig. 2**



2

3